
SOME OBSERVATIONS ON THE FINE STRUCTURE OF THREE GENERA IN THE TETRASPORACEAE^{1, 2}

DANIEL E. WUJEK

Department of Biology, Wisconsin State University, La Crosse, Wisconsin 54601

ABSTRACT

Detailed study of the ultrastructure of three members of the Tetrasporaceae, *Paulschulzia pseudovolvox*, *Schizochlamys gelatinosa*, and *Tetraspora gelatinosa*, has revealed, for the first time, that the pseudocilia of two of the genera possess a 9+0 fibrillar structural arrangement. *Schizochlamys gelatinosa* was found to lack thylakoids traversing the pyrenoid. The pyrenoids of the other two genera were found to resemble more closely those described for other green algae.

Recently both Wujek and Chambers (1965) and Lembi and Herndon (1966) have reported on the fine structure of the pseudocilia of two *Tetraspora* species. The present study reports observations on species of the three genera *Tetraspora*, *Paulschulzia*, and *Schizochlamys*, all members of the Tetrasporaceae.

MATERIALS AND METHODS

Paulschulzia pseudovolvox (Schulz) Skuja was obtained from the Culture Collection of Algae at Indiana University (#167; Starr, 1964). The species was

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also observed and isolated from collections made in Harvey County, Kansas. *Schizochlamys gelatinosa* A. Br. and *Tetraspora gelatinosa* (Vauch) Desv. were collected from habitats in the vicinity of Lawrence, Kansas. All algae were cultured in modified Bristol's solution (Deason and Bold, 1960), to which soil-water extract had been added.

Cells were harvested by centrifugation and fixed in a variety of fixatives: 2% KMnO₄, buffered (Luft, 1956) or unbuffered, 1% buffered OsO₄ (Palade, 1952), or 6% buffered glutaraldehyde (Sabatini, et al., 1963) and post fixed in 1% buffered OsO₄ for times ranging from 45 to 90 minutes. Materials were dehydrated in a standard series of acetone or ethanol/propylene oxide and were embedded in araldite-epon mixture #1 (Mollenhauer, 1964). Sections were obtained with glass knives on an LKB Ultratome and examined with an RCA 3F electron microscope.

OBSERVATIONS

Paulschulzia—The broadly ellipsoid colonies (fig. 1) contain two to eight cells or daughter colonies within a mucilage envelope. Each cell bears two long pseudocilia (to 200 μ length; usually 75–100 μ), which project beyond the outermost investment of the colony. When stained they may be broader in the basal than in the distal portion. The basal bodies of the pseudocilia are inserted at the anterior end of each cell. With electron microscopy, an H-shaped structure is evident (fig. 2). A contractile vacuole is located near the basal body (fig. 2). Figure 3 shows this relationship with a closed contractile vacuole. Cross sections of the cylinder have not revealed the 9-pointed star known to be present in other algae (Manton, 1965), which possesses true cilia (flagella) with the 9+2 arrangement.

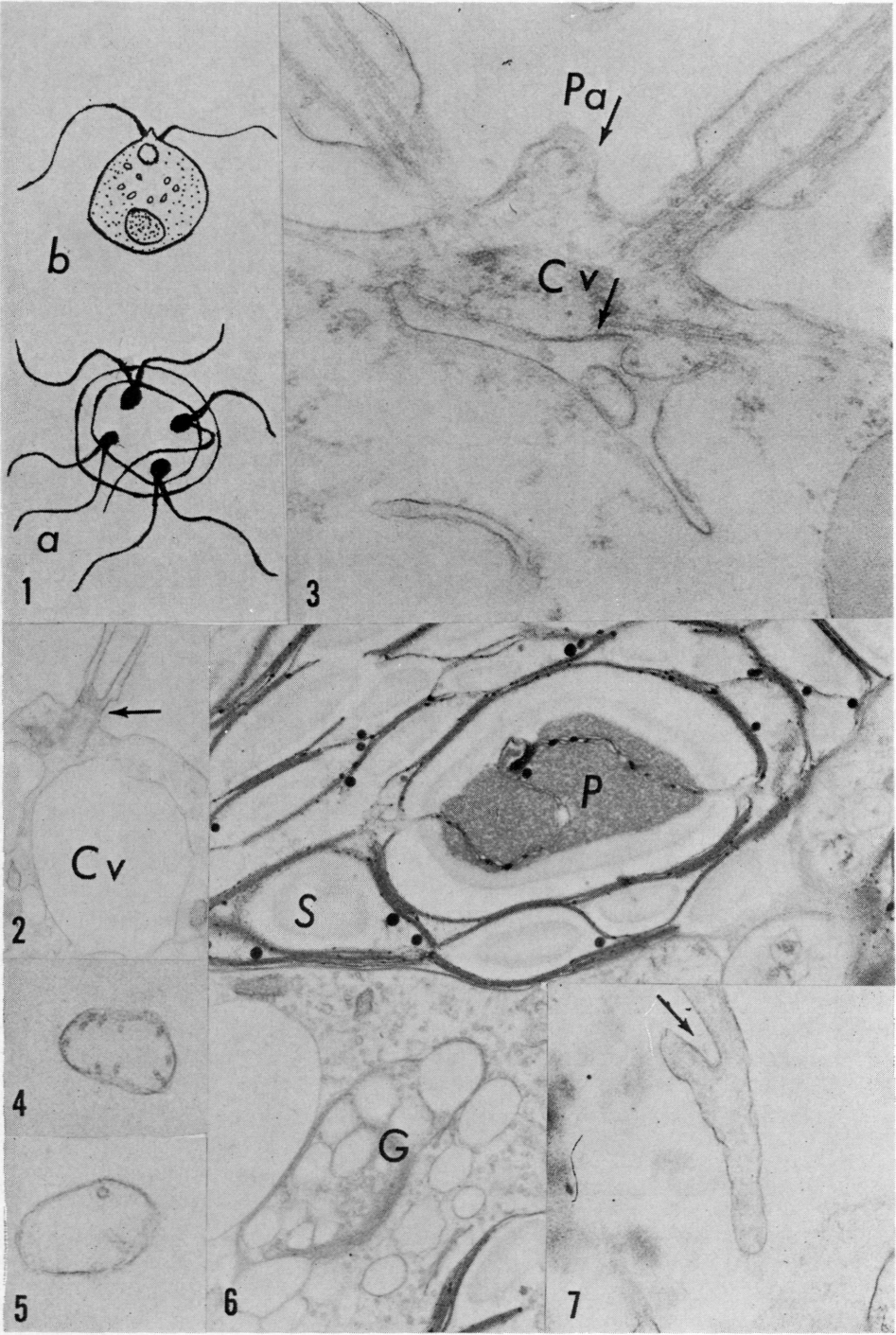
In sections distal to the basal body, the number of fibrils making up the pseudocilium diminish so that, at its greatest length, only two fibrils closely appressed to the limiting cytoplasmic membrane are present (fig. 4, 5). Fibrillar roots as observed in *Tetraspora lubrica* (Lembi and Herndon, 1966) were not observed in this genus. Other structures of the pseudocilium are similar to those already described in *Tetraspora* (Wujek and Chambers, 1965; Lembi and Herndon, 1966).

Located basally in the parietal, cup-shaped chloroplast is the pyrenoid. The pyrenoid is traversed by double discs (fig. 6). The thylakoids of the pyrenoid have the same dimension as those of the chloroplast. Although in some cases oil and lipid bodies were seen close to the chloroplast envelope (and pyrenoid), a significant association between the two is not well established.

Schizochlamys—In working out the life history of *S. gelatinosa*, Thompson (1956) found the spherical cells of this genus to be united usually into amorphous colonies, with each cell containing a massive parietal chloroplast. He further observed that intermediate and mature colonies possessed pseudocilia. The pseudocilia in this genus differ from other members of the family in that, in addition to having simple pseudocilia, many cells bear pseudocilia that fork (fig. 7), so that each cell apparently bears two to eight pseudocilia. The ultrastructure of these pseudocilia does not differ in any way from those previously described.

EXPLANATION OF FIGURES 1-6

FIGURES 1-6. *Paulschulzia pseudovolvox*. Fig. 1. A diagram. a. A four-celled colony with pseudocilia projecting beyond the colony matrix. $\times 250$. b. Individual cell with papilla. $\times 950$. Fig. 2. Longitudinal section of a pseudocilium base showing H-shaped cylinder (arrow). $\times 31,500$. Fig. 3. Longitudinal section showing a closed contractile vacuole (Cv) at the pseudocilia bases (arrow). A papilla (Pa) is also indicated. $\times 44,000$. Fig. 4. Cross section of a pseudocilium above the cylinder showing nine single fibrils. $\times 39,900$. Fig. 5. Pseudocilium distal from papilla showing two single fibrils. $\times 88,000$. Fig. 6. Longitudinal section of a mature cell with densely packed starch (S), Golgi body (G), and a pyrenoid (P). $\times 16,000$.



As in *Paulschulzia*, no roots could be observed connecting the two basal bodies or extending into the peripheral cytoplasm as in *Tetraspora lubrica* (Lembi and Herndon, 1966). The region of the basal body proximal to the cylinder is composed of nine triplet fibrils, as in flagella, having the 9+2 fibril arrangement.

A pyrenoid is present in *Schizochlamys* (fig. 8), with the structure appearing as an "outpocketing" (Gibbs, 1962; Joyon, 1963) from the chloroplast. In some specimens, two pyrenoids were observed, although this may have been due to the orientation of the plane of sectioning. The pyrenoids were without thylakoids traversing them, although the bounding chloroplast membranes could be observed around them. A large number of sections showing detached pyrenoids were observed. In a dividing cell, the pyrenoid remained visible, unlike that observed in *Scenedesmus* (Bisalputra and Weier, 1964). No examples of quadripartition of the protoplast, as reported elsewhere (Smith, 1950), were observed.

Tetraspora—No new data on the pseudocilia of *T. gelatinosa* were obtained. The pyrenoid in this genus is similar to that found in *Paulschulzia*. The pyrenoid does not disappear during cell division, as reported in other Volvocales (Overton, 1889), but rather precedes division by its duplication (fig. 9).

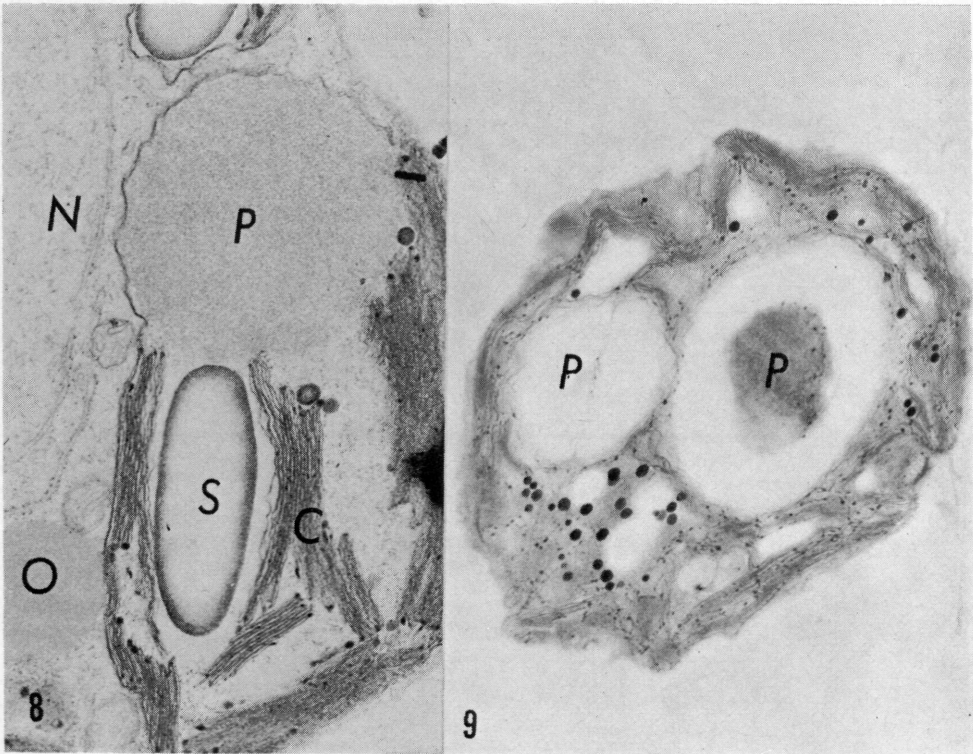


FIGURE 7. *Schizochlamys gelatinosa*. Forking of a pseudocilium (arrow). $\times 47,000$.

FIGURE 8. *Schizochlamys gelatinosa*. Section showing a pyrenoid (P) without any traversing thylakoids. $\times 29,600$.

FIGURE 9. *Tetraspora gelatinosa*. Section of a cell undergoing division with two pyrenoids (P) present. $\times 13,200$.

DISCUSSION

A consistent and in some ways unique pattern of cell fine structure has been found in this examination of three genera of the Tetrastropaceae. Basal structure of the pseudocilia and the presence of mitochondria in this region suggest some relationship to true flagella (Lembi and Herndon, 1966). The chloroplast envelope consists of two membranes, unlike the three membranes which have been reported for the Phaeophyta and Chrysophyta (Bouck, 1965; Manton, 1957; Wujek, 1966). Although it has been reported (Smith, 1950) that cells of *Schizochlamys* do not contain both starch and oil, all the cells of this study that contained oil also contained at least some starch.

In spite of the basic similarities among the taxa studied, one organelle was different, this being the type of pyrenoid. *Tetraspora* and *Paulschulzia* exhibited the pyrenoid type most common to the green algae, while the pyrenoid of *Schizochlamys* was similar to those described for several of the Chrysophyceae (Joyon, 1963; Wujek, 1966). An examination of other members of the family may confirm that this type of pyrenoid is the exception rather than the rule.

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